In the present study pharmacological activities like antimicrobial activity, antifungal activity and antioxidant activity were determined in the selected herbal plants. In this study garlic, onion, ginger, lemon and turmeric were taken for the study. It is well known fact that herbal plants have good pharmacological properties. Garlic, onion, turmeric, lemon and ginger are common herbal spices which are generally used in Indian kitchen. These herbal plants have good antimicrobial nature which is revealed and supported by many researchers. Lemon has very good antioxidant activity and turmeric has very good antifungal and anti-inflammatory activity. In this study, herbal medicinal plants i.e. Garlic, Onion, Ginger, Lemon and Turmeric were also taken in different combinations to assess level of various enzymatic and non-enzymatic antioxidant properties, antifungal and antimicrobial properties. Main focus of this study was to assess various combination of juice extracts of herbal spices which can show much better antibiotic and antioxidant properties than standard antibiotics or antioxidants used. Some selected combinations of extracts have shown very good antibiotic and antifungal properties, which is higher than the reference standard antibiotics taken. Another finding of this study is related to Anti-Inflammatory activities shown by some combinations of juice extracts of herbal plants. Combination of turmeric, ginger & garlic in 1:1:1 ratio have shown more anti-inflammatory activity as compared to Diclofenac-Sodium taken as standard reference.

**Antimicrobial Study :-**

From the results it can be observed that Garlic juice extract is most potent than the other herbal plants against most of bacteria. Garlic juice extract produced the highest zone of inhibition against *B. cereus* (20.8 mm) followed by *Micrococcus spp* (20.2mm) & *S. aureus* (19.5 mm). The order of bacteria which were inhibited more strongly by the garlic juice extracts are-

*B. cereus* > *Micrococcus spp* > *S. aureus* > *Kleibsella spp* > *E. coli* > *Proteus spp*
From the observation, *B. cereus* was found to be the most sensitive bacteria to garlic juice and the garlic juice showed greater potency than the Chloroamphenicol which was used as reference against the bacteria.

The author Cavallito C. J & Bailey J. H, 1951 described that garlic contains the allicin (sulphur containing compound) inhibits the growth of both gram negative and gram positive bacteria, which is main antimicrobial agent in the garlic juice.

**Onion** juice extract could inhibit *B. cereus* (14.5 mm), *S. aureus* (13.1) & *Micrococcus spp* (13.0) and other bacteria could not be inhibited. Onion produced less zone of inhibition than the reference antibiotic-chloroamphenicol.

**Lemon** juice extracts showed the highest zone of inhibition of *S. aureus* (17.6 mm) followed by Micrococcus spp (16.1 mm). Lemon was not found to be effective against *Proteus spp* bacteria. The order of bacteria which were inhibited more strongly by the lemon juice extracts:

S. aureus > Micrococcus spp > E. coli > B. cereus > Kleibsella spp

**Turmeric** juice extracts showed the highest zone of inhibition of Micrococcus spp (18.2 mm) followed by B. cereus (17.5 mm). The order of bacteria which were inhibited more strongly by the turmeric extracts:

Micrococcus spp > B. cereus > S. aureus > *Proteus spp* > E. coli > Kleibsella spp

Ginger juice extracts singly produced the highest zone of inhibition against *B. cereus* (19.6 mm) followed by Micrococcus spp (18.2 mm). The order of bacteria which were inhibited more strongly by the its extracts:

*B. cereus* > Micrococcus spp > *Proteus spp* > *S. aureus* > E. coli > Kleibsella spp

The result agrees with the claim made by Elestin K.L; 1997, according to the author, lemon juice and oil are effective in killing germs and it is taken as prevention of stomach infection.

Overall from this study it can be observed that Garlic juice has maximum potential of antibacterial resistance followed by turmeric, ginger and lemon. Onion out of the selected plants has shown minimum antibiotic potential.
**In Combination Study**

From the double combination results shown in table 4.2.b & c, it can be observed that double combination of garlic with turmeric & ginger (GT&GGi) are having much better antibacterial potential as compared to other combinations. These combinations not only have more bacterial resistance than reference antibiotic (chloroamphenicol) but also more than that of individual juice extracts.

Combination of garlic & lemon (GL) produced the highest zone of inhibition (20.6 mm) against bacteria *E. coli* followed by GGi (combination of Garlic & Ginger) & GT (garlic and turmeric). GL produced 50% more zone of inhibition as compared to reference compound (antibiotic-chloroamphenicol) and about 25% more than any singly applied juice extract (garlic). It may be due to the combined induced effect of active constituent present in the extracts.

Against the bacteria *Klebsella spp*, combination of GT (garlic and turmeric) & GGi (garlic and ginger) produced highest zone of inhibition 20.2 mm and 19.9 mm respectively. OL combination was not effective against *Klebsella spp*.

Against the bacteria *Proteus spp*, GL combination (20.3 mm) followed by GO (18.6 mm) were most effective. Combinations of GT (18.3 mm) & GGi (18.2 mm) were also close to GO inhibition. LGi is not effective while OL combinations has shown least inhibition against these bacteria. GL combination has shown about 30% more inhibition than individual effect.

Against the bacteria *S. aureus*, GGi combination (20.0 mm) followed by GT (19.9 mm) were most effective. Combinations of GL (19.6 mm) is also close to GT. OL is not effective against these bacteria. GGi combination has shown about 10% more inhibition than individual effect.

Against the bacteria *B. cereus*, GT combination (22.5 mm) followed by GGi (22.1 mm) were most effective. Combinations of GiT (21.9 mm) is also close to GGi effect. All combinations were effective against these bacteria. GT combination has shown about 15% more inhibition than individual effect.

Against the bacteria *Micrococccus spp*, GiT (22.3 mm), GGi (21.7 mm), GT (21.7 mm) have shown more effectiveness and resistance. The reference- chloroamphenicol has
shown 20.0mm zone of inhibition. Almost all double combinations are potent against these bacteria.

Overall in double combinations most potent is GT combination (22.5 mm) against B. cereus. In double combination study, Micrococcus spp bacteria are most sensitive.

MIC - The minimum inhibitory concentration (MIC) of the garlic, Lemon, Turmeric & Ginger juice extracts was found to be 40 µl/ml, 45 µl/ml, 60 µl/ml & 65 µl/ml respectively against E. coli while for onion juice, MIC was very high, so could not be detected in it. MIC of chloroamphenicol was found to be 155 µg/ml in the study.

The minimum inhibitory concentration (MIC) of the Garlic, Lemon Turmeric & Ginger juice extracts was found to be 30 µl/ml, 80 µl/ml 70 µl/ml & 65 µl/ml respectively against Klebsella spp bacteria. While for Onion juice, MIC was very high, so could not be detected in it. MIC of chloroamphenicol was found to be 130 µg/ml in the study. Overall against this selected bacteria garlic concentration required is minimum because it is most effective.

Antifungal activities the plant juice extracts - applied singly (results in Table 4.2, a)

Garlic juice extracts produced the highest zone of inhibition of 25.5 mm against fungi A. terreus followed by P. expansum (20.7 mm). The order of fungi which were inhibited more strongly by the garlic juice extracts follows as:

A. terreus > P. expansum > A. niger > A. japonicus

Ginger extracts also produced the zone of inhibition against fungi A. terreus.

Onion juice extracts produced zone of inhibition of 12.1 mm against fungi A. niger. Onion juice extracts could not inhibit other fungi in this study.

Turmeric extracts produced the highest zone of inhibition of 18.0 mm against fungi P. expansum A. followed by A. Japinicus (16.8 mm) followed by A. terreus (16.5 mm).

Lemon juice extracts produced the highest zone of inhibition of 16.0 mm against fungi A. terreus followed by P. expansum (15.9 mm). Lemon juice extracts could not inhibit other fungi in this study.

Ginger juice extracts produced the highest zone of inhibition of 17.9 mm against fungi A. terreus followed by P. expansum (16.6 mm). Ginger juice extracts could not inhibit A. niger fungi in this study.
If we analyse the data with reference to selected fungi, then it can be observed that Garlic juice extract is having maximum potential of antifungal activity. Its juice inhibited all fungi with maximum zone of inhibition except in case of *A. Jepinicus* fungi. Ginger juice extract was found to be more effective against *A. Jepinicus fungi*. Garlic has shown more anti-inflammatory effect than the reference anti-fungal drug ketacanazole. with the help of zone of inhibition, it can be predicted that garlic is more antifungal potent than ginger & others.

**Antioxidant Activity**

It is known fact that antioxidants are required to suppress the free radicals like reactive oxygen species are generated in the body

**SOD**

The specific Activity of superoxide dismutase (SOD) in the lemon was observed maximum i.e. 23.0 unit/mg protein as compared to turmeric (20.1 unit/mg protein) followed by garlic (18.5unit/mg protein ). SOD activity of onion is very lessas compared to other plant activities. The order of the plants having high amount of SOD value is:

Lemon>Turmeric> Garlic > Ginger > Onion

**Specific Activities of enzymatic Antioxidant (Catalase)**

Catalase enzymatic antioxidant activity was determined in the selected plant juice extracts, results are shown in table4.3.2

The specific activity of catalase in the Turmeric rhizome were found to be highest i.e. 262 unit/mg protein followed by 255 unit/mg by lemon. Garlic juice specific activity is 210unit/mg protein while that of Ginger is 110 and onion’s specific activity is 40.2 unit/mg protein. So, it can be observed that specific activity of superoxide dismutase in highest in lemon but catalase activity is highest in the turmeric rhizome which is higher than other plants. Onion,s bulb has less specific activity than turmeric and ginger. From the result and observation, the order of the plants having high amount of Catalase values is-

Turmeric >Lemon>Garlic > Ginger> Onion
Glutathione peroxidase (GPx) activity

The activity of peroxidase in the selected plants are different from one another. The highest activity is found in fruits of lemon i.e. 250 IU/L followed by turmeric rhizome i.e. 240 IU/L and Ginger i.e. 219 IU/L. Garlic bulbs has shown 190 IU/L activity and at the last is onion i.e 135 IU/L. so, from the result and observation, the order of the plants having high amount of peroxidase activity

Lemon > Turmeric > Ginger > Garlic > Onion

The order of Glutathione peroxidase was not same order as in case of Peroxidase activity. i.e Turmeric > Lemon > Garlic > Ginger > Onion

Non enzymatic antioxidant (Vitamin C)

Antioxidant activities were also determined by non-enzymatic antioxidant method. Determination of vitamin C (Ascorbic acid) is very common non-enzymatic antioxidant method. Results of ascorbic acid assays are shown in table 4.3.4. Beta-carotene bleaching method and thiocyanate assays methods are also used to assess antioxidant activities.

Vitamin C was found highest in lemon fruits i.e 3.5 mg/gm of sample followed by turmeric i.e. 2.90 mg/gm and followed by Ginger i.e. 2.7 and then in Garlic i.e. 2.6 mg/gm, while onion has shown minimum 2.1 mg/gm vitamin C. The Antioxidant Activity of fresh plant is higher than the dry ones. So, the turmeric and Garlic’s fresh juice have much higher antioxidant activity than the onion.

This finding is in accordance with other authors (Elestin K.L.; 1997) who reported that lemon & turmeric have high level of Vitamin C content that helps to improve resistance to infection, making it valuable for colds and flu. It is taken as preventive for many conditions including stomach infection, circulatory problems and arteriosclerosis. The order of the plant having high amount of Vitamin C:

Lemon > Turmeric > Ginger > Garlic > Onion.

Reactive oxygen species from both endogenous and exogenous source may be involved in the etiologies of such diverse human diseases as arteriosclerosis, cancer and neurodegenerative diseases, as well as in the processes like inflammation and ageing.
**β-carotene bleaching method** - Antioxidant activity by β-carotene bleaching method was determined by taking 10 & 20 μL of essential oils. Results are given in table-4.3.5, in this method, Lemon has shown maximum activity followed by mousambi, orange and pine apple. Similar trend was obtained when concentration of essential oil was increased up to 20μL. When other selected plants were assessed for antioxidant activity by this method then we observed that after the lemon, turmeric is having more antioxidant property followed by ginger, galic and onion. Lemon is having about double antioxidant property as compared to turmeric. When concentrations of plant extracts are doubled then antioxidant activities increase by slight margin of about 10%, so 10μL concentration for antioxidant activity is sufficient.

**Thiocyanate Antioxidant Activity**

By this method also once again lemon has shown max antioxidant property. Second best was shown by mousambi among the citrus fruits. Among the selected plants we can observe that turmeric is having more anti-oxidant property than ginger, garlic and onion by this method. Obviously citrus fruits have more anti-oxidant properties, however turmeric and ginger are also having good anti-oxidant properties.

**Anti-Inflammatory Activity**

Anti inflammatory activity was determined by Human Red Blood Cell (HRBC) membrane stabilization method. This HRBC method has been used as an in-vitro method to study the anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane. This method has been used for evaluation of in-vitro anti-inflammatory activity by many researchers like Rajurkar R(2009), Gandhidasan R(1991) etc. The main action of anti-inflammatory agent is to inhibit the cyclooxygenase enzymes (COX-1 & COX-2) which are responsible for the conversion of Arachidonic acid to prostaglandins. Results of Anti-inflammatory activity determined by Human Red Blood Cell (HRBC) are shown in table 4.4.

Anti-inflammatory activity in the juice extracts of selected plants i.e. garlic, onion, turmeric, ginger & onion were determined by using in-vitro HRBC method. In this
method % inhibition was determined. Individually turmeric has shown maximum inhibition of about 63% at concentration of 50 microgm/ml followed by ginger (59% inhibition) and then garlic juice which has shown 47% inhibition. Onion and lemon have inhibited about 40% at same concentration. When plant extract concentration was increased to 100 µg/ml, then % inhibition was increased by about 10-12%. Overall turmeric has shown best anti-inflammatory effect while standard reference compound Diclofenac sodium has shown about 80% inhibition at moderate concentration of 50 microgm/ml.

**Overall in this study** on the basis of different observations it can be concluded that –

- Individually garlic is most potent herbal plant against the selected bacterial strains among the selected plant.
- *Micrococcus spp* bacteria are observed to be most sensitive against these selected herbal plant extracts.
- In double combinations, GT (garlic & turmeric), GGi (garlic and Ginger) and GiT (garlic & turmeric) combinations were observed to be very effective against most of the selected gram-positive and gram negative bacteria. They have also shown more potential as compared to standard antibiotic-chloroamphenicol.
- In triple combination, GGiT (garlic, ginger & turmeric) has shown best antibacterial potential than any other combination and this is also more than standard compound. Other potent combinations are GOT and GOL.
- Garlic individually has shown best anti-fungal potential followed by ginger and turmeric.
- In combinations, GT (garlic & turmeric) combination is having best anti-fungal potential followed by GGi (garlic and Ginger). They were found to have more potential as compared to standard antifungal-ketcanazole.
- In triple combination GGiT (garlic, ginger & turmeric) is best having anti-fungal potential. This combination was found to have more potential as compared to standard antifungal-ketcanazole.
- Overall lemon is having best anti-oxidant properties.
• In combinations, LT (lemon and turmeric), and GiT (ginger & turmeric) combinations are better to show antioxidant properties.

• Turmeric is observed to have best anti-inflammatory activity.

• In combinations, GiT (ginger & turmeric) and GT (garlic & turmeric) have shown very good anti-inflammatory activity which is nearly same as by standard anti-inflammatory agent diclofenac-sodium.

• So by this study we get many combinations of herbal plants which show and possess much better anti-microbial, antifungal, anti-oxidant and anti-inflammatory properties.